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# Experiments on Visual Cells by Nature and Man: In Search of Treatment for Photoreceptor Degeneration

## *Friedenwald Lecture*

Mark O. M. Tso

The primary purpose of the eye is light perception, a process which is initiated in the photoreceptor cells. These cells are well sheltered in the center of the eye, protected structurally by the sclera, nourished by the richly vascularized uvea and safeguarded by the blood-retinal barrier of the retinal pigment epithelium. Yet the leading cause of blindness in the United States is age-related photoreceptor degeneration. Our lack of understanding of the pathologic processes and pathogenetic mechanisms of photoreceptor degeneration coupled with the scarcity of comparable animal models result in the unavailability of effective treatment of photoreceptor degeneration.

In this lecture I attempt to provide an overview of pathogenetic factors of photoreceptor degeneration as well as the alterations of the visual cells and their cellular environment in photoreceptor degeneration. After studying a number of experimental models of photoreceptor degeneration, I came to the conclusion that photic retinopathy would be a revealing and informative model. A therapeutic approach to photoreceptor degeneration was developed based on the experimental model of photic retinopathy.

### **Pathogenetic Factors of Photoreceptor Degeneration**

Photoreceptor degeneration is a complicated multifactorial process. While the pathogenetic mecha-

nisms of many forms of photoreceptor degeneration are not definitively known, a number of relatively well defined clinical entities exhibit photoreceptor degeneration. It seems appropriate to begin our search of pathogenetic mechanisms by first reviewing human examples of photoreceptor degeneration. Unfortunately, tissues from humans are frequently obtained only at the end stage of the disease. Additional pathogenetic factors may have acted directly or indirectly on the retina and obscured the primary event, and multifactorial intervention is not uncommon in the human diseases. It is therefore important to discern primary and secondary events of photoreceptor degeneration.

### **Viral Retinopathy**

A number of viruses, such as herpes simplex, herpes zoster, and cytomegaloviruses, may infect the retina and produce severe retinitis. The photoreceptor cells degenerate as part of the inflammatory process. However, the lesions in measles maculopathy<sup>1-6</sup> appear different. They are usually located in the macula and the posterior pole and follow a chronic degenerative course. The macular degeneration may precede other neurologic symptoms of subacute sclerosing panencephalitis (SSPE). SSPE affects mostly school-age children and is characterized by intellectual deterioration, inappropriate behavior, periodic myoclonic movement of extremities and trunk, coma, and death. The disease usually follows naturally occurring measles (rubeola), but has been reported to occur after immunization against rubeola. Fifty percent of patients with SSPE may have involvement of the visual system. Ophthalmoscopically, the macula appears swollen, occasionally with radial folds, and subsequently develops into an atrophic pigmented scar with little clinical evidence of an inflammatory process. The fundus findings have been misinterpreted as juvenile macular degeneration. Pathologic studies of the early retinal lesions demonstrated mild mononuclear cell infiltration

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Supported in part by National Institutes of Health research grants EY-1903 and EY-6761; by training grant EY-07038 and core grant EY-01792 from the National Eye Institute, Bethesda, Maryland; by United States Army Medical Research Grant DAMD 17-89-2-9025; by research grants from Lions of Illinois Foundation, Chicago, Illinois; and by the Renée Izenman and Morris Korzen Memorial Foundation.

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with specific degeneration of the cone cells<sup>5</sup> (Fig. 1). In the late phases of the disease, the retina was reduced to a glial membrane. Landers and Klintworth<sup>1</sup> identified intranuclear microtubules, characteristic of measles virus in retinal cells. Font et al<sup>4</sup> observed the presence of measles virus in infected retinal cells by immunofluorescence and also found intranuclear and cytoplasmic viral nucleocapsids.

Besides measles maculopathy, another photoreceptor degeneration with a viral cause is scrapie retinopathy.<sup>7-10</sup> Scrapie is a slow virus causing a degenerative disease of the nervous system in adult sheep and goats. Scrapie, together with kuru and Creutzfeldt-Jakob disease, are infectious subacute spongiform encephalopathies characterized by an afebrile course, and leading to dementia and death. The scrapie agent can be transmitted to hamsters and other rodents by intracerebral inoculation and in these animals results in a degenerative retinopathy. This degeneration begins in the outer segments of the visual cells, progresses slowly to the inner segments, and results in thinning of the outer nuclear layer.<sup>7,8</sup> The pathologic process is not unlike that of atrophic age-related macular degeneration in humans. The possibility that other slow viruses may be involved in various forms of photoreceptor degeneration in humans needs further investigation.

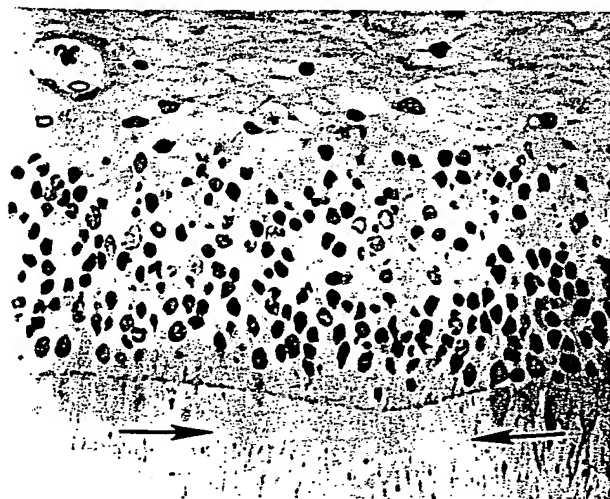


Fig. 1. Retinal lesion in subacute sclerosing panencephalitis. A 9-year-old white girl complained of difficulty in seeing at distance. Visual acuity was 20/20 in the right eye and 20/200 in the left eye. One year later she developed myoclonic spasm, became comatose and died. Retinal lesion in the posterior pole of right eye showed focal loss of cone inner and outer segments (arrows) with preservation of inner and outer segments of rod cells (toluidine blue,  $\times 260$ ). From LaPiana F, Tso MOM, and Jenis FH: *Ann Ophthalmol* 6:603, 1974, by permission.

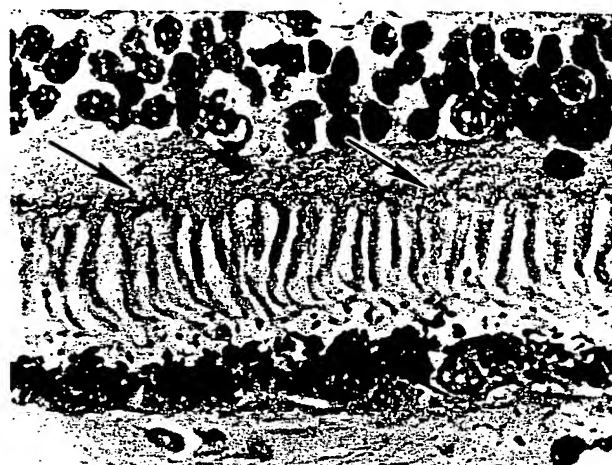


Fig. 2. Paraneoplastic retinopathy. A 72-year-old black woman complained of rapid, progressive loss of vision and achromatopsia. Visual acuity was 20/80 in both eyes. A few months later an endometrial carcinoma was detected. Section of the retina showed total loss of cone cells. Note the absence of cone nuclei lying on the external limiting membrane (arrows) (hematoxylin-eosin,  $\times 250$ ). From Cogan DG, Kuwabara T, Currie J, Kattah G, and Harper D: Meeting of the Eastern Ophthalmic Pathology Society, 1986, by permission.

### Paraneoplastic and Autoimmune Retinopathies

Paraneoplastic retinopathy is a syndrome characterized by degeneration of the retina, specifically involving the photoreceptor cells in patients with neoplasms in other organ systems of the body and without direct metastatic invasion of the eye.<sup>11-17</sup> The visual symptoms may precede the diagnosis of the primary malignancy. The patients complain of diminished visual acuity, photopsia, ring or central scotomas, achromatopsia, or nyctalopia. The electroretinogram is usually flat. A patient with paraneoplastic retinopathy, studied by Buchanan et al,<sup>13</sup> showed marked constriction of the visual fields to 2° from fixation, but had normal color vision as evaluated by pseudoisochromatic plates. Histopathologically, the macular cone and rod cells remained, whereas photoreceptors peripheral to the macula had totally disappeared. A patient studied by Cogan et al<sup>17</sup> had bilateral central scotomata and achromatopsia. Pathologic study of the eyes showed extensive loss of cone cells throughout the retina with preservation of rod cells (Fig. 2). The pigment epithelium was atrophic and partially depigmented. The choriocapillaris was normal.

The primary malignancies reported in paraneoplastic retinopathy vary, and include oat cell carcinoma of the lung, endometrial carcinoma, infiltrating ductal carcinoma of the breast, and others. Paraneoplastic involvement of the retina is not unique. A

carcinomatous neuromyopathy secondary to oat cell carcinoma of the lung (Eaton-Lambert syndrome) is believed to be partially induced by a circulating protein released from the oat cell carcinoma.<sup>18</sup> Carcinomatous neuropathy also has been described in Guillian-Barré syndrome. Involvement of the neuromuscular junctions in thymoma leads to myasthenia gravis.

The pathogenetic mechanisms of paraneoplastic retinopathy have been the subject of intense investigation. Autoimmunity has been implicated in some patients with this form of photoreceptor degeneration. Two of the patients described in the literature responded to steroid therapy.<sup>12,14</sup> Thirkill et al<sup>16</sup> observed antiretinal antibodies in four patients with paraneoplastic retinopathy. The serum immunoglobulins bound to an antigen of molecular weight 23 kD in pooled normal retina. Of the two cases of paraneoplastic retinopathy reported by Grunwald et al,<sup>15</sup> the serum antibody in one patient bound to retinal proteins at 65 and 20 kD, and the serum antibodies of the second patient showed binding to retinal proteins at 205 and 145 kD. They suspected that the retinal antigen might be related to the triplet proteins of neurofilaments.

It has been proposed that autoimmune mechanisms may play a role in a variety of retinal degener-

ations,<sup>19</sup> including retinitis pigmentosa, central serous retinopathy, and age-related macular degeneration. Antibodies against two of the neurofilament triad proteins with molecular weights of 145 and 205 kD have been noted in the serum of patients with retinitis pigmentosa.<sup>20</sup> Recent studies in our laboratory<sup>21</sup> identified immunoreactive antibodies to human retinal proteins in the serum of 14 of 30 patients with age-related macular degeneration. These serum antibodies demonstrated positive binding to a doublet retinal protein of molecular weight between 58 and 62 kD. The localization of these antigens in the outer segment was confirmed by indirect immunofluorescent labeling of human retinas (Fig. 3). Furthermore, the serum antibodies cross-reacted with the lower band of the purified bovine neurofilament protein of 68 kD, suggesting that this protein may also belong to the neuronal cytoskeleton elements. Research is in progress to explore this hypothesis further.

Autoimmunity experiments with a number of the retinal proteins have been performed in a variety of experimental animals, including guinea pigs, rats, rabbits, birds, and monkeys.<sup>19</sup> Wong and colleagues induced an autoimmune retinitis in monkeys by either homologous outer segments or rhodopsin.<sup>22-24</sup> Even though inflammatory cells were identified in

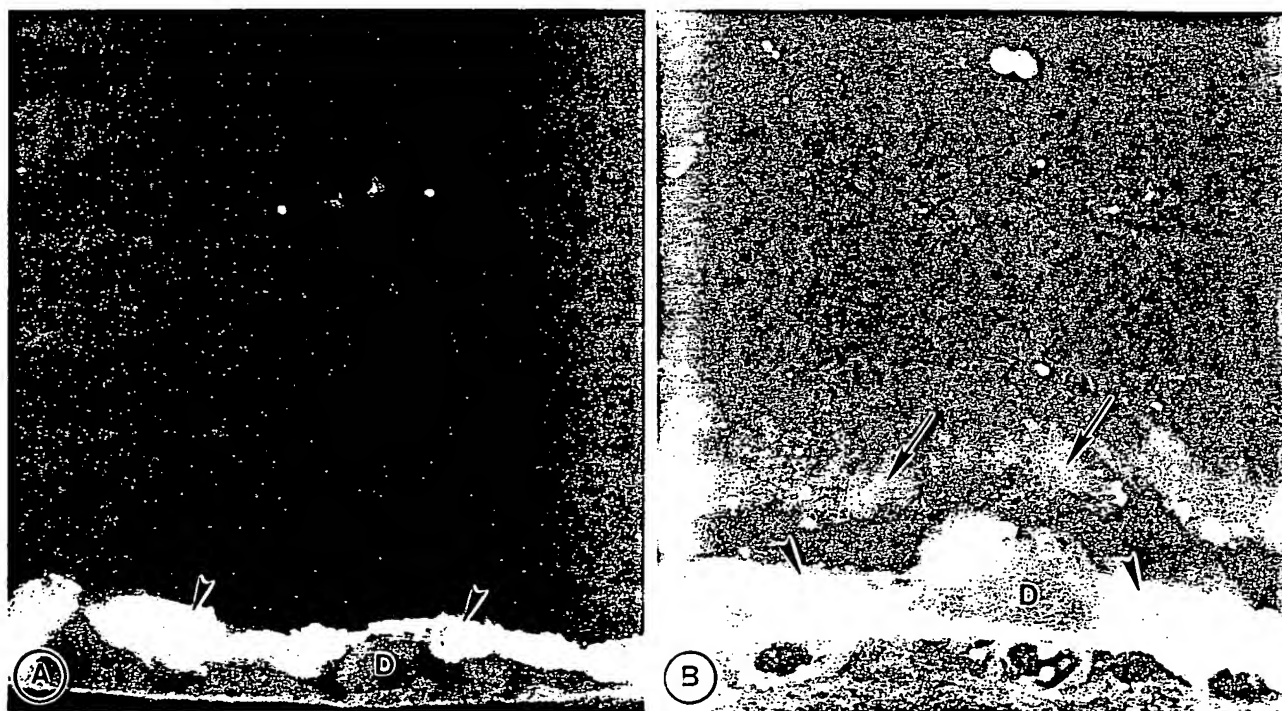


Fig. 3. Indirect immunofluorescent labeling of normal human retina incubated with serum of (A) normal human subjects and (B) patient with age-related macular degeneration. Note in (B): Strong immunolabeling in outer segments (arrows) of photoreceptor cells. Moderate labeling was also seen around the photoreceptor nuclei and outer plexiform layer. Autofluorescence of lipofuscin granules in the pigment epithelium (arrowheads). D indicates drusen in the normal aged retina.

the early stages of the disease, chronic rhodopsin sensitization at a low dosage resulted in loss of photoreceptor cells without much inflammatory reaction, as seen in various forms of human retinal degeneration.

The proteins of the retina are highly immunogenic, and autoimmunity may play an important part in photoreceptor cell degeneration. However, the precise role of these antiretinal antibodies in the serum of patients with photoreceptor degeneration is unclear. The detection of antiretinal antibody in serum may be a primary or secondary event. To prove an autoimmune causation in a disease, Witebsky's criteria may be applied.<sup>25</sup> These include (1) demonstration of circulating antibodies; (2) identification of corresponding autoantigens; (3) induction of autoreactivity against these antigens in an experimental model; (4) pathologic alteration similar to the human disease; and (5) transfer of the disease by transferring antibodies or lymphocytes. Even though autoimmune mechanisms have been suggested for many degenerative diseases of the central nervous system, only myasthenia gravis has met all of Witebsky's postulates.

#### Choroidal Ischemia Affecting Photoreceptor Cells

The retinal vasculature supplies the inner layers of the retina, and the choroidal vasculature nourishes the outer layers of the retina external to the middle limiting membrane, including the entire photoreceptor cells and pigment epithelium. Occlusion of the choroidal vasculature may lead to degeneration of photoreceptor cells. However, the angioarchitecture of the choroidal vasculature shows marked regional variations, resulting in different patterns of choroidal ischemia and photoreceptor degeneration. In the posterior pole, the sinusoidal choriocapillaris radiate from the central supplying arterioles and are drained by the postcapillary venules in a lobular pattern.<sup>26,27</sup> This system facilitates efficient and fast blood flow. In addition, interarterial and intervenous shunts are present in the posterior choroid. Therefore, small focal choroidal ischemia may be compensated for, without photoreceptor cell degeneration. In contrast, in the peripheral choroid, the arterioles and venules run parallel to the ora serrata, and the choriocapillaris connects the precapillary arterioles to the postcapillary venules at right angles in a ladder pattern. As a result, occlusion of the peripheral choroidal arterioles frequently causes a triangular whitening of the retina with loss of photoreceptor cells.

In an acute generalized occlusion of choriocapillaris, such as seen in posterior ciliary arteritis, thrombocytopenic purpura, and disseminated intravascular coagulopathy, extensive photoreceptor cell loss may

be seen.<sup>28</sup> But if the occlusion is slow in onset, compensatory mechanisms may develop in the choroidal circulation and so prevent degeneration of photoreceptor cells. Such compensatory change was demonstrated in a patient who had extensive amyloid deposit occluding the entire choriocapillaris of the posterior pole, yet in whom photoreceptor cells and pigment epithelium remained intact.<sup>29</sup>

In age-related macular degeneration, the choriocapillaris undergoes atrophic changes. The normal sinusoidal choriocapillaris in the macula are replaced by a tubular capillary network (Fig. 4).<sup>30</sup> This disturbance of the angioarchitecture of the choroidal blood flow and its relationship to the photoreceptor cell degeneration need further exploration.

#### Hereditary Degeneration of Photoreceptor Cells

A large number of photoreceptor degenerations are known to be hereditary in nature, even though, in many instances, the genetic defect has not been clearly defined. Most hereditary photoreceptor degenerations have definitive clinical, ophthalmoscopic, and electrophysiologic features, and may affect a selective region of the fundus where the disease may first occur. The preferential regional involvement helps the clinician to arrive at the diagnosis of the disease and may influence the clinical manifestation. In a pathologic study of cone-rod dystrophy,<sup>31</sup> a diffuse loss of photoreceptor cells was seen predominantly in the macular and peripheral retina, while some of the cone and rod cells in the equator were well preserved (Figs. 5, 6). Because of this preferential regional involvement, the ratio of degenerated cone cells to remaining cone cells was greater than the degenerated rod cells to remaining rod cells. This observation partially accounts for the greater reduction of electroretinographic amplitudes for cone as opposed to rod functions. This regional loss of photoreceptor cells differs from that of retinitis pigmentosa, in which photoreceptor loss traditionally starts in and is most severe in the equatorial region.

It is difficult to pinpoint the locus of the initial cellular pathology in human retinal degenerations because of the complex interaction between the tissues in the photoreceptor-pigment epithelium-choroid complex. Blodi<sup>32</sup> attempted to classify this group of diseases by the primary sites of involvement of the tissues, including the choroid (eg, central areolar choroidal sclerosis), Bruch's membrane (Doyle's honeycombed choroiditis), retinal pigment epithelium (Best's disease), and neuroretina (Stargardt's disease). In recent years, tissues from better examples of different types of hereditary photoreceptor degenerations have been obtained, and pathologic features of





Fig. 4. (A) Flat preparation of choriocapillaris of normal human posterior pole showing lobular pattern. The choriocapillaris radiates from a central arteriole (single arrow), and is drained into peripheral venule (double arrows) (periodic acid-Schiff,  $\times 80$ ). (B) Choriocapillaris in a patient with age-related macular degeneration showing tubular capillary network (arrows) (periodic acid-Schiff,  $\times 80$ ). From Tso MOM: *Ophthalmology* 92:628, 1985, by permission.

some of these entities are now more clearly defined. For example, Eagle et al<sup>33</sup> studied an eye with well documented Stargardt's disease and observed that the retinal pigment epithelium was filled with an abnormal form of lipofuscin. As tissues from early stages of

various hereditary photoreceptor degenerations become available for pathologic study, the primary locus of these diseases may be differentiated from the secondary degeneration.

Morphologic features of the hereditary retinal degenerations, such as the overloading of pigment epithelium with phagosomes and lipofuscins, may be a secondary rather than primary defect (Fig. 6).<sup>31</sup> In other instances, such as the inherited retinopathy in colliers,<sup>34</sup> the photoreceptors were seen to produce outer segments in a disoriented fashion toward the synaptic terminals (Fig. 7) and were likely to be the primary cellular event. Other pathologic features may be more generalized rather than specific to certain diseases. Tubulovesicular degeneration of outer segments is seen in photic injury, retinitis pigmentosa, cone-rod dystrophy, and other retinopathies. Filamentous bundles in the perikaryon of photoreceptor cells in hereditary macular degeneration in the guinea baboons<sup>35</sup> simulates those seen in degenerating cone cells of Alaskan Malamutes or retinitis pigmentosa in humans.<sup>36</sup> The genetic makeup of the photoreceptor cells and the interaction with their environment play an important part in the manifestations of these hereditary diseases.

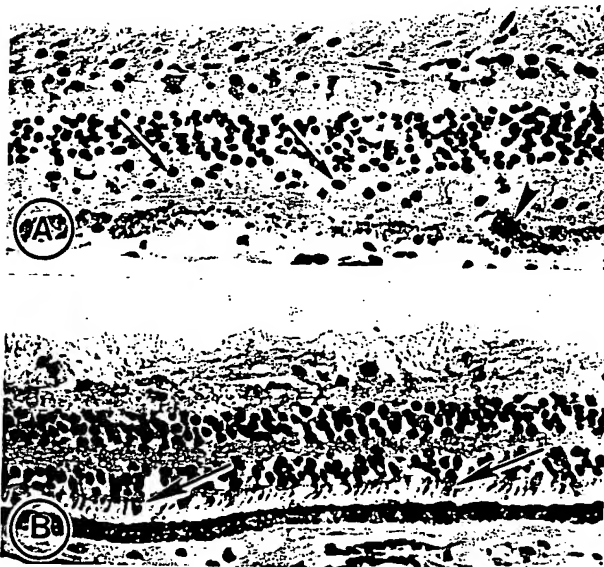


Fig. 5. (A) Macula of a patient with cone-rod dystrophy. The outer nuclear layer (arrows) had largely disappeared. Pigment-laden macrophages (arrowhead) were noted in the subretinal space. The retinal pigment epithelium appeared irregularly pigmented (eosin,  $\times 80$ ). (B) Equatorial retina of the same patient. The outer nuclei layer was reduced from 2-3 nuclei thick, but had both rod and cone cells (arrows) and their inner and outer segments. The retinal pigment epithelium was of normal height but contained lipofuscinlike granules (hematoxylin-eosin,  $\times 180$ ). From Rabb MF, Tso MOM, and Fishman GA: *Ophthalmology* 93:1443, 1986, by permission.

### Physical Injury to Photoreceptor Cells

Even though the photoreceptor cells are securely protected in the interior of the eye, these cells are readily accessible to light because their primary function is light detection. As a result, the most frequently encountered physical injury of the photoreceptor cells is photic injury. However, nature does provide a number of mechanisms to protect the photoreceptor

Fig. 6. Equatorial retina of patient with cone-rod dystrophy showing marked loss of photoreceptor nuclei (P). Expanded cytoplasmic processes of Müller cells (Mu) filled the outer nuclear layer. The external limiting membrane consisted of cell junctions between the many Müller cells and a few photoreceptor cells. Both cone and rod elements were seen. The photoreceptor lamellae appeared irregular (arrow). The retinal pigment epithelium showed abundant lipofuscinlike granules (L) in the base of the cells, with melanin granules (M) aggregating in the apical region of the cells. BM indicates Bruch's membrane ( $\times 5900$ ). From Rabb MF, Tso MOM, and Fishman GA: *Ophthalmology* 93:1443, 1986, by permission.



cells from light injury. The ocular media, including the cornea, aqueous, lens, and vitreous, filter most of the emission at the ultraviolet spectrum. However, after cataract extraction or other forms of surgical intervention, some of these protective barriers may be removed so that the photoreceptor cells are prone to be damaged by other spectra of radiant energy. The mechanisms by which the photoreceptive cells and their associated cellular systems remove excessive light energy reaching the retina are not fully understood. A portion of the light energy is absorbed by the melanin granules of the pigment epithelium and then

dissipated into the surrounding structure. The blood flood of the choriocapillaris has been proposed to have a cooling effect by removing excessive energy absorbed by the pigment epithelium. Nature also gives the photoreceptor cells other cellular protective mechanisms to protect from photic injury by providing antioxidants to neutralize the free radicals generated by light. In addition, nature provides humans with excessive numbers of photoreceptor cells such that, when there is a loss of 56% of the neuronal channels, visual acuity of 20/20 still can be maintained.<sup>37</sup> Despite all of these protective mechanisms,



Fig. 7. A disoriented photoreceptor cell in an inherited retinopathy of a 30-day-old Collie, showing outer segments (arrow) developing toward the outer plexiform layer (OPL) in the posterior pole. P indicates synaptic terminals ( $\times 10,000$ ). From Santos-Anderson RM, Tso MOM, and Wolf ED: *Invest Ophthalmol Vis Sci* 19: 1281, 1980, by permission.

photic injury to the retina remains a real threat to vision. This is discussed in detail in the following section.

Mechanical disturbance to photoreceptor cells includes commotio retinae, traumatic displacement of photoreceptor cells, and retinal detachment. Sipperly et al<sup>38</sup> showed that in traumatic retinopathy, the pathologic changes in the disrupted photoreceptor cells accounted for the opaque retina seen in commotio retinae. No vascular leakage was seen. The response of the retinal pigment epithelium to this traumatic injury was comparable to that in an experimental retinal detachment and in light-induced retinal damage. Cogan<sup>39</sup> postulated that in traumatic retinopathy, the pigment epithelium releases an enzyme that destroys the photoreceptor cells. Specific examples of traumatic retinopathy are illustrated in a later section.

#### Age-Related Photoreceptor degeneration

Age-related macular degeneration is one of the commonest causes of blindness in the United States. Many hypotheses have been proposed to explain its pathogenesis. A careful review of the literature shows that many etiologic factors are involved.<sup>40</sup> Hereditary

influence, nutritional deficiency, immunologic disorders, cardiovascular and respiratory diseases, preexisting eye diseases, photic injury, and other causes have been implicated.

Advancing age appears to be the most significant factor. The prevalence of the disease dramatically increases after the age of 65 years and reaches a prevalence of 27.9% among both men and women aged 75–85 years. In some studies, a higher prevalence among women was noted, but Klein and Klein,<sup>41</sup> on analyzing the data from the National Health and Nutritional Examination Survey, noted no significant increase in prevalence among females. Similarly, the prevalence of the disease was reported to be lower in blacks than in whites of comparable age, but Klein and Klein<sup>41</sup> showed a comparable prevalence among whites and blacks in the same age group. Other personal characteristics of age-related macular degeneration include family history of the disease, weakness of hand grip, and short stature. The weak hand grip might represent physiologic aging that results from muscular atrophy and weakening body strength. Specific ocular characteristics associated with the disease are hyperopia and blue irides. Systemic diseases significantly correlated with macular degeneration include systemic hypertension and a history of chronic



lung infection. Paetkau and co-workers<sup>42</sup> noted that the mean age of onset of blindness in one eye due to age-related macular degeneration in smokers was 64 years in contrast to 71 years in nonsmokers. Hyman et al<sup>43</sup> noticed the association of smoking with macular degeneration only in men. Other clinical characteristics associated with age-related macular degeneration include drusen and atrophy of the choriocapillaris. A possible relation of chronic photic insult with age-related macular degeneration is discussed later.

The above pathogenetic factors of photoreceptor degeneration do not necessarily act alone, but rather exert themselves in different combinations on the visual cells, producing varying patterns of clinical manifestations. Therefore, the age of onset of symptoms, the progression of the disease, and the severity of degeneration differ among patients. Our challenge is to sort out the respective roles of these various pathogenetic factors in the degenerative diseases of the photoreceptor cells.

### Alterations of the Photoreceptor Cells and Their Cellular Environment in Photoreceptor Degeneration

The photoreceptor cell is a long, slender cell extending from the retinal pigment epithelium to the middle limiting membrane. It consists of an outer and an inner segment, the perikaryon, the axon, and the synaptic terminal. It is surrounded by the Müller cell and is in contact with the retinal pigment epithelium and the bipolar and horizontal cells. While the pathologic changes of the photoreceptor cells vary with the types of degeneration depending on the etiologic factors, a general pattern of the degeneration of the visual cells is observed. In degeneration of the photoreceptor cells, the Müller cells and the retinal pigment epithelium are also deranged. The disruption of the bipolar and horizontal cells in photoreceptor degeneration is not yet clearly defined and is under active investigation in our laboratory. The following section discusses the pathology of the visual cells and the disturbance of their cellular environment in photoreceptor degeneration.

### Pathologic Changes of Photoreceptor Cells

To illustrate the morphologic patterns of visual cell degeneration, examples are taken from age-related macular degeneration (Fig. 8). In the earliest stage of the disease, the retinal pigment epithelium appears thin and atrophic. The overlying photoreceptor cells are unremarkable. As the degenerative process progresses, the outer segments of the photoreceptor cells are shortened, so that the inner segments of the photoreceptor cells approximate the retinal pigment epi-

thelium. Still later, the inner segments disappear with the external limiting membrane approaching the retinal pigment epithelium. In the final stage, the photoreceptor nuclei disappear. This morphologic staging of the photoreceptor degeneration in age-related macular degeneration does not imply that the retinal pigment epithelium initiates the ensuing process. Indeed, in occasional cases of age-related macular degeneration, the photoreceptor nuclei disappear, and the pigment epithelium appears normal in height but is loaded with lipofuscin granules. A similar pattern of cellular degeneration is frequently seen in hereditary, viral, traumatic, photic, and other retinopathies.

The disappearance of the outer segments as one of the early manifestations of photoreceptor degeneration suggests that the complicated cellular process of photoreceptor outer segment production is impaired, or that shedding of the outer segments is greatly accelerated. Increased phagocytosis by retinal pigment epithelium is another possibility. Excessive accumulation of lipofuscin phagosomes within the retinal pigment epithelium has been postulated to play a role in age-related macular degeneration.<sup>44,45</sup> However, it must not be interpreted that the loss of outer and inner segments equates with the loss of visual function. Electrophysiologic signals have been recorded from photoreceptor cells that have lost their inner and outer segments.<sup>46</sup>

The patterns of the photoreceptor cell death appear in several other forms. Some of the degenerating photoreceptor nuclei, as in patients with retinitis pigmentosa<sup>37</sup> and photic injury, exhibit a densified chromatin and cytoplasm and resemble "type-B dark cells."<sup>47</sup> In other photoreceptor cells, as seen in the inherited macular degeneration of guinea baboons,<sup>35,36</sup> the perikaryon exhibited hydropic degeneration, showing swelling of the endoplasmic reticulum with watery cytoplasm. The significance of this form of degeneration is not determined. In still other instances, such as in photic retinal injury in rats,<sup>48</sup> the photoreceptor nuclei and cytoplasm dissolve with few cellular remains. The mechanisms of dissolution are currently being studied in our laboratory. Definitive apoptosis of the photoreceptor cells,<sup>47</sup> as characterized by compaction and margination of nuclear chromatin to form sharp circumscribed masses and condensation of cytoplasm, is also being considered and is under active investigation at our laboratory.

### Interdependency of Photoreceptor Cells and Retinal Pigment Epithelium

In photoreceptor degeneration, the retinal pigment epithelium is frequently affected. This is not surprising, since the retinal pigment epithelium regulates the

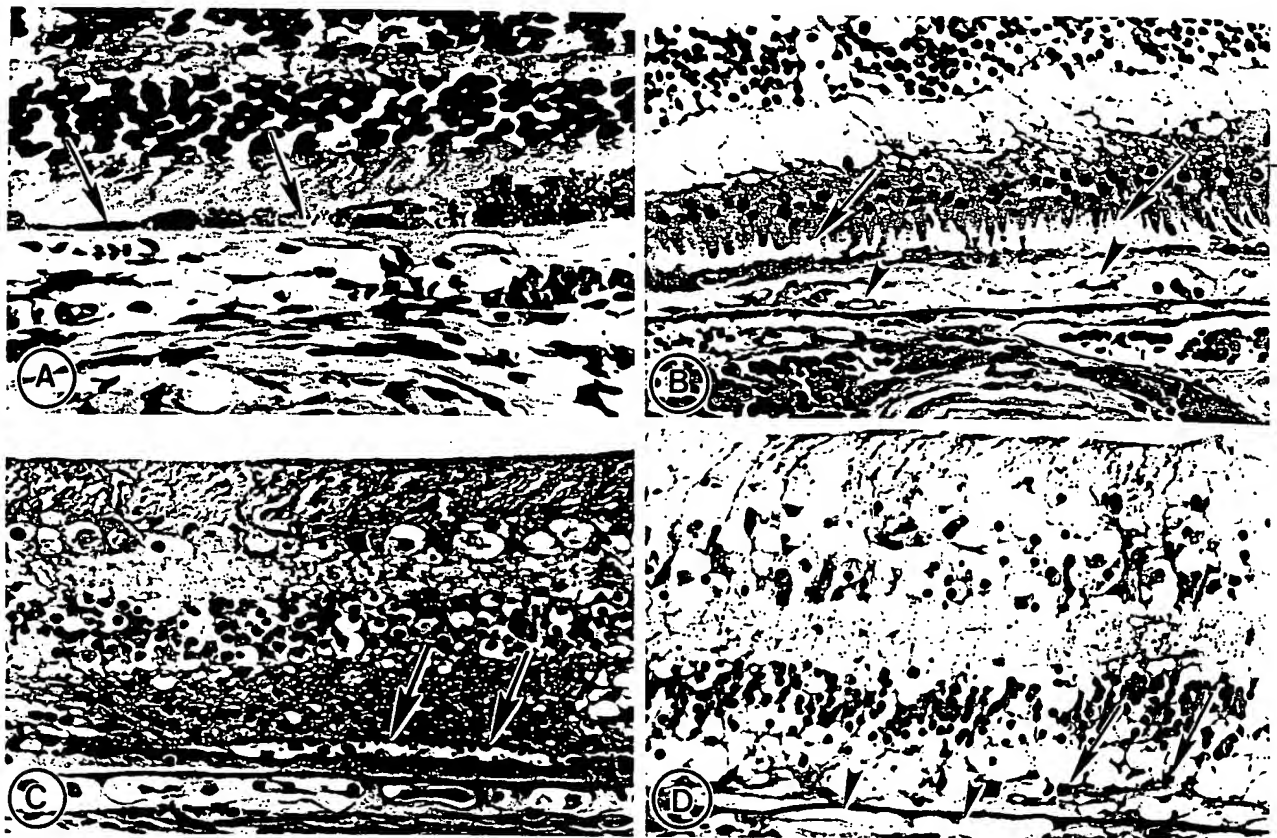


Fig. 8. Photoreceptor degeneration in age-related macular degeneration. (A) In the early stage of the disease, the retinal pigment epithelium (arrows) appeared thin and atrophic but photoreceptor inner and outer segments appeared unremarkable (hematoxylin-eosin,  $\times 350$ ). (B) Moderate photoreceptor degeneration showed shortening of outer segments (arrows) of photoreceptor cells and the inner segments approximated pigment epithelium. Note the presence of a subretinal pigment epithelium neovascularization net (arrowheads) (hematoxylin-eosin,  $\times 350$ ). (C) Moderately advanced stage of photoreceptor degeneration showing loss of both inner and outer segments. The external limiting membrane (arrows) approximated pigment epithelium, which was markedly atrophic (hematoxylin-eosin,  $\times 280$ ). (D) Advanced stage of age-related macular degeneration showing few nuclei of the photoreceptor cells (arrow). The retinal pigment epithelium had largely disappeared from Bruch's membrane (arrowheads) (hematoxylin-eosin,  $\times 250$ ).

nutritional and metabolic needs of the photoreceptor cells; provides a blood-retinal barrier; gives mechanical and physiologic support; and removes the outer segment discs of rods and cones. However, the disruption of the photoreceptor-retinal pigment epithelium relationship does not necessarily lead to photoreceptor cell death, as illustrated in an experimental traumatic retinopathy (Fig. 9). In that study, some of the photoreceptor cells in the macula of a monkey were displaced into the inner layers of the retina. In the new environment of the outer plexiform layer, the photoreceptor cells were deprived of the support of the retinal pigment epithelium but appeared to survive, and maintained inner and outer segments 6 months after the injury.

In another example, a patient with atrophic age-related macular degeneration had marked atrophy of the retinal pigment epithelium associated with displacement of the photoreceptor cells in the macula from the outer nuclear layer into the subretinal space

(Figs. 10, 11). The perikaryon of some of the displaced photoreceptor nuclei lay on bare Bruch's membrane, with inner and outer segments facing the internal limiting membrane. The remaining photoreceptor cells in the outer nuclear layers maintained their inner and outer segments in a normal direction facing the choroid. As a result, two rows of photoreceptor cells, both of which were deprived of pigment epithelium, had opposing inner and outer segments, but appeared to survive. The above two examples illustrate that displaced photoreceptor cells may survive. It is not surprising that photoreceptor cells surgically transplanted into the subretinal space may live for a period of time, but that neural connection of these cells may be difficult.

The retinal pigment epithelium provides the blood-retinal barrier for the photoreceptor cells, but disruption of this barrier does not lead to death of the photoreceptor cells. This argument is supported by the study of the macula of a monkey exposed to the



Fig. 9 (A) Macula of a monkey with experimental traumatic retinopathy, showing displaced photoreceptor cells (arrows) into the outer plexiform layer (toluidine blue,  $\times 250$ ). (B) Electron microscopy showing a displaced photoreceptor cell with inner and outer segments (arrowheads) connected by a cilium (arrow) ( $\times 6000$ ).

light of an indirect ophthalmoscope for 2 hr. Five years later a subretinal scar of proliferated retinal pigment epithelium had formed, which leaked of fluorescein diffusely.<sup>49</sup> Horseradish peroxidase tracer study demonstrated the passage of tracer through the retinal pigment epithelium into the subretinal space (Fig. 12). Photoreceptor cells with normal inner and outer segments approximated the plaque of proliferated pigment epithelial cells. These visual cells appeared to be morphologically intact, and had survived despite disruption of the blood-retinal barrier for 5 years. In the same context, the normal functioning photoreceptor cells of the pineal organ of the frog do not have the support even of a retinal pigment epithelium. The outer segments of these photoreceptor cells are surrounded freely by cerebrospinal fluid and thrive in the absence of retinal pigment epithelium. The mammalian ocular photoreceptor cells must have adapted to biochemical, physiologic, and metabolic needs different from those of the pineal of frog.

### Interdependency of Photoreceptors and Müller Cells

The photoreceptor perikaryon is completely surrounded by the Müller cells, which provide metabolic, mechanical, and structural support to the photoreceptor cells. The Müller cells are responsible for synthesizing and inactivating a number of neurotransmitters in the retina; for initiating the synthesis and removal of some retinoids and cellular retinal binding proteins; and for regulating the extracellular potassium level of the retina. Friedenwald et al demonstrated that the Müller cells also have phagocytic functions and can remove debris from the vitreous cavity and transport them into the subretinal space.<sup>50</sup>

In a number of diseases, the photoreceptor-Müller cell relationship is disturbed. In an experimental model of occlusion of central retinal artery and vein, the cells in the inner nuclear layer,<sup>51</sup> including the Müller cells, died, but the photoreceptor cells being nourished by the choroidal circulation survived (Fig.

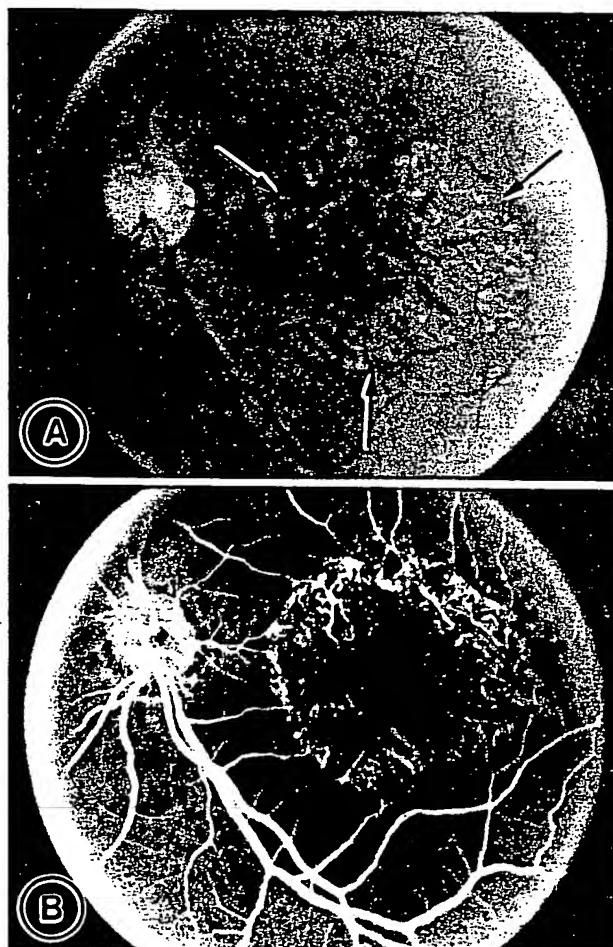


Fig. 10. Fluorescein angiogram of a patient with atrophic age-related macular degeneration showing (A) thinning of the retina and loss of pigment epithelium and choriocapillaris (arrows) and (B) filling of large choroidal vessels. There was absence of pigment epithelium and choriocapillaris.

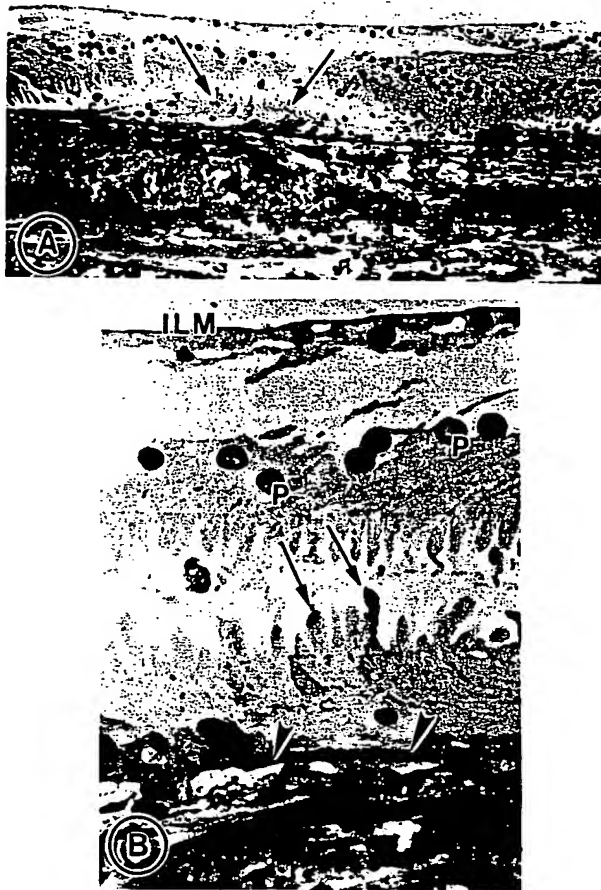


Fig. 11. (A) Macula of patient in Figure 10 showing displacement of photoreceptor cells into the subretinal space (arrows) (toluidine blue,  $\times 50$ ). (B) Under higher magnification, note the absence of pigment epithelium. The displaced photoreceptor nuclei lay on bare Bruch's membrane (arrowheads) with inner and outer segments (arrows) facing the internal limiting membrane (ILM) and opposite the inner and outer segments of the remaining photoreceptor cells (P). Both layers of photoreceptor cells were deprived of pigment epithelium (toluidine blue,  $\times 350$ ).

13). Morphologic study showed that the photoreceptor cells were attached to each other without the intervening Müller cell processes. These photoreceptor cells appeared to survive without the support of adjacent Müller cells 3 weeks after the experimental vascular occlusion.

In retinoblastoma with photoreceptor differentiation,<sup>53</sup> the tumor cells developed inner and outer segments (Fig. 14). However, no Müller cells were differentiated between the tumor cells. The absence of Müller cells did not prevent the differentiation of cone inner and outer segments by the tumor photoreceptor cells.

In photoreceptor degenerations inflicted by viral, autoimmune, ischemic, hereditary, aging, or physical

insults, the degenerative process most frequently starts at the outer and inner segments. However, the visual cells can survive years without inner and outer segments in chronic retinopathies. The retinal pigment epithelium and Müller cells are vital for their normal functions of visual cells, but their absence does not necessarily result in photoreceptor cell death. In search of therapy for photoreceptor degeneration, it is imperative to discern the crucial factors of maintenance and regeneration of inner and outer segments and the critical mechanisms which prevent eventual cell death.

### Photic Retinopathy as an Experimental Model To Study Photoreceptor Regeneration

Few histopathologic studies of various macular and retinal degenerations in humans have been performed. When these tissues were obtained (often fortuitously), the disease was usually in an advanced stage and the retinal architecture was extensively disturbed. It has been difficult to reconstruct the complete course of the disease. To obtain human retinal tissues in different stages of degeneration by multiple retinal biopsy is impractical. As a result, extensive search of animal models of photoreceptor degeneration, dysplasia, or dystrophy has been undertaken.<sup>54</sup> Examples of retinal diseases studied in animal models have included photoreceptor degeneration in the guinea baboon, miniature poodle, and Wag/Rij rat; photoreceptor dysplasia in the Irish Setter, Collie, rod mouse, rds mouse, Norwegian Elkhound, and Alaskan Malamute; and pigment epithelium-choroid diseases in RCS rats, central progressive retinal atro-

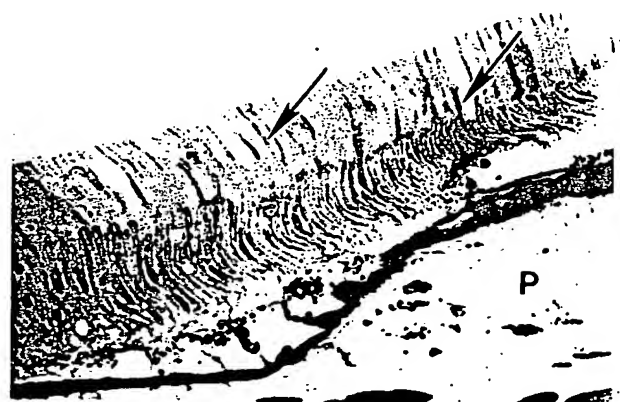


Fig. 12. The macula of a monkey exposed to the light of an indirect ophthalmoscope for 2 hr, 5 years earlier. A subretinal proliferated pigment epithelium (P) was formed. Horseradish peroxidase tracer (arrows) passes through the pigment epithelial plaque into the subretinal space. Photoreceptor cells with intact inner and outer segments stood on the pigment epithelial plaque (unstained,  $\times 350$ ). From Tso MOM and Woodford BJ: Ophthalmology 90:952, 1983, by permission.



Fig. 13. Retinal ischemia following simultaneous occlusion of central retinal artery and vein in a rhesus monkey. The external limiting membrane (XLM) was formed by photoreceptor cells joining adjacent photoreceptor cells with intervening Müller cell processes ( $\times 1100$ ). (Inset A) Light microscopic appearance of the photoreceptor elements fanning out (thin arrows) from the external limiting membrane (thick arrows) in the form of a fleurette arrangement (toluidine blue,  $\times 615$ ). (Inset B) Note a photoreceptor cell adhering to an adjacent photoreceptor cell without intervening (arrows, subretinal space) Müller cell ( $\times 12,000$ ). From Juarez CP, Tso MOM, Wichard AJ, vanHeuven P, Hayreh MS, and Hayreh SS: *Int Ophthalmol* 9:89, 1986, by permission.



phy in dogs, and chorioretinal gyrate atrophy and mucopolysaccharidosis in cats. These animal models have provided useful insight into human retinal degenerations. However, many morphologic, physiologic and biochemical features of the human retina are unique, and comparable models of nonhuman primate retinopathy are few. After evaluating a number of different animal models, I concluded that photic retinopathy is a revealing experimental model for retinal degeneration.

Radiant energy may damage the retina by three mechanisms: mechanical, photocoagulative, and photic. In mechanical injury, the retina is disrupted by acoustic transients in gaseous formation. In photocoagulative injury, the inflicting light generates a high temperature, resulting in coagulation of the retinal proteins. In photic injury, light of the visible spectrum initiates certain photochemical reactions and brings about degenerative changes in the retina without significantly elevating the temperature. In

this section, our discussion concentrates on photic retinopathy.

Photic retinopathy provides an experimental model in which we can study the degenerative, reactive, and the reparative phases of retinal degeneration in a variety of subjects, including rodents, guinea pigs, monkeys, and humans, so that species specificity may be explored. The advantage of studying photic retinopathy in monkeys is clear: the anatomy, physiology, biochemistry, and pathology of their retina very closely resembles those of the human retina (Fig. 15). Photic retinopathy in the rodent model is also useful because rodents can be bred easily so that the genetic factors are controlled. Sampling of retinal tissues at various stages of the disease can be achieved easily. The rodent model also affords us an opportunity to do a semiquantitative evaluation of the pathologic process with statistical analysis. The rat retina contains virtually only rods, however, and so retinal degeneration involving cone cells cannot be studied.

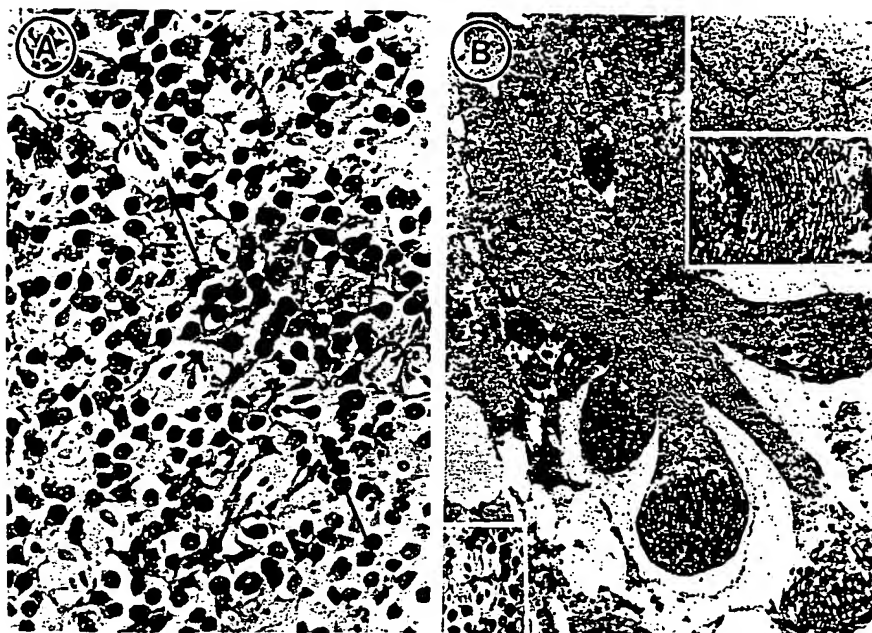


Fig. 14. Retinoblastoma with photoreceptor cell differentiation. (A) Tumor photoreceptor elements in the fleurettes (arrows). (B) Electron microscopy of a cluster of fleurettes showing that the photoreceptor elements were joined to adjacent cells with the zonula adherens cell junction without differentiation of Müller cells in between ( $\times 5000$ ). Lower left inset shows a cluster of fleurettes in light microscopy ( $\times 440$ ). Upper right insets showing cross and tangential sections of the cell attachment between the photoreceptor elements without intervening Müller cells ( $\times 16,000$ ). (A) From Tso MOM: *Trans Am Acad Ophthalmol Otolaryngol* 74:959, 1970, by permission. (B) From Tso MOM, Fine BS, and Zimmerman LE: *Am J Ophthalmol* 69:350, 1970, © the Ophthalmic Publishing Company, by permission.

The use of photic retinopathy as a model to study retinal photoreceptor degeneration has other advantages. The causal relationship between retinal photic injury and macular degeneration has aroused much interest.<sup>55</sup> Young and Droz described the continual

renewal of the photoreceptor outer segments of both rod and cone cells.<sup>56</sup> If the efficiency of this system of physiologic renewal is altered by excessive light exposure, repetitive photic injury may provide a clue to the senescence of photoreceptor cells. Interestingly,

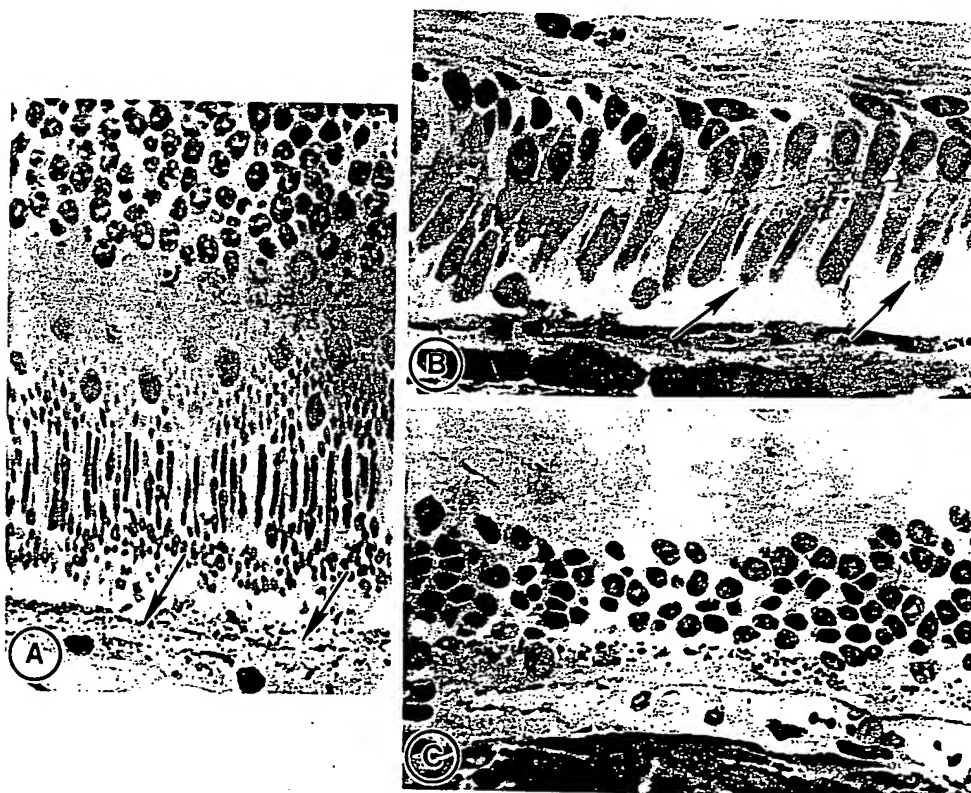


Fig. 15. (A) Retina of a monkey 7.5 months after exposure to the light of an indirect ophthalmoscope for 30 min. The retinal pigment epithelium (arrows) remained irregularly depigmented, but the inner and outer segments of the photoreceptor cells appeared aligned (toluidine blue,  $\times 600$ ). (B) The retina of a normal monkey 7 1/2 months after exposure to the light of an indirect ophthalmoscope for 2 hr, showing atrophic retinal pigment epithelium. The outer segments of the photoreceptor cells never regenerated. The inner segments (arrows) of photoreceptor cells approached retinal pigment epithelium. The outer nuclear layer was reduced to 2 or 3 nuclei thick (toluidine blue,  $\times 600$ ). (C) Retina of scurvy monkey 8 months after exposure to the light of an indirect ophthalmoscope

for 2 hr. The photoreceptor cells lost both inner and outer segments, and the nuclei of the photoreceptor cells approached pigment epithelium (toluidine blue,  $\times 600$ ). From Tso MOM: *Trans Am Ophthalmol Soc* 85:498, 1987, by permission.

photoreceptor cells in rhesus monkeys that had suffered photic injury showed persistent shortened outer segments 5 years after light exposure, not unlike those seen in age-related macular degeneration.<sup>49</sup> Gartner and Henkind<sup>57</sup> noted that photoreceptor cells in the aging human retina decreased in number. Similar observations were noted in monkeys that have been exposed to excessive light. It has been suggested that age-related macular degeneration is related to chronic repetitive mild photic injury to the retina over a lifetime.<sup>55</sup> Despite the circumstantial evidence of the relationship between age-related macular degeneration and photic insult, two frequent precursors of macular degeneration—drusen and serous detachment of retinal pigment epithelium—have not been experimentally produced by photic injury. Furthermore, the subretinal pigment epithelial neovascularization net produced by photic injury in nonhuman primates lacks the aggressive growth characteristics seen in the human disciform macular degeneration.<sup>49</sup> Other factors must also play a part in the pathogenesis of age-related macular degeneration in humans.

#### Experimental Photic Maculopathy in Humans

A number of reports have described the deleterious effect of environmental lighting on human vision. United States Navy personnel exposed to sunlight for 3–4 hrs/day for 2 weeks were noted to have temporarily elevated night vision thresholds that returned to normal after the eyes were protected from sunlight for 1 day.<sup>58</sup> In contrast, other personnel, wearing protective polarizing sunglasses with 12% transmission, had significantly lower night vision thresholds. Smith<sup>59</sup> reported gradually decreased visual acuity and macular pigmentary changes in military personnel stationed on a tropical island in the Pacific Ocean during World War II after working outdoors for 4 months or longer. Such pigmentary changes were not found in yeomen and pharmacists who worked indoors on the island.

It has long been known that directly gazing at the sun or solar eclipse can result in photic maculopathy.<sup>60–62</sup> Ewald and Ritchey described a yellow "exudate" that appeared deep in the fovea in the acute stage after sun-gazing.<sup>62</sup> The fovea developed a deep red discoloration with a fine granular pigmentation and a ring of coarse pigmented aggregate 2 weeks later. A lamellar or full-thickness macular hole with a honeycomb pattern was seen in the late stage. Penner and McNair described a gradual loss of central vision after sun-gazing, with subsequent recovery.<sup>61</sup>

In order to study the clinicopathologic process in photic maculopathy in humans, four patients who had malignant melanoma of the choroid in one eye

and who were scheduled for enucleation agreed to gaze at the sun shortly before enucleation.<sup>63,64</sup> The refractive error of the affected eyes was corrected with lenses, and the contralateral eyes were covered during the experiment. Before sun-gazing, the visual acuity of the patients ranged from 20/30–20/15, and fluorescein angiography showed no abnormality. All patients were free of pain during the exposure, and none had difficulty in fixating on the sun. They described that the sun appeared initially as a bright red ball and then turned black with a pink halo. Two other patients who looked at the sun with an undilated pupil detected no central scotoma on tangent screen or Amsler grid, and their visual acuity was unchanged. Two patients who stared at the sun with a dilated pupil described a relative central scotoma. One of them had a mild loss of visual acuity from 20/20–20/25 within 2 days after the exposure. Ophthalmoscopically, the affected fovea in 3 patients appeared mildly swollen and was minimally discolored. Fluorescein angiography, however, showed a foveal leakage of dye in the late venous phase 24 hr after exposure in all patients (Fig. 16). The recovery time on photostress tests on all four patients was remarkably prolonged. Three eyes were enucleated within 2 days after sun-gazing. The fourth patient, whose enucleation was delayed until 12 days after exposure, suffered a continuous deterioration in visual acuity, from 20/20 to 20/40. Irregular pigmentation developed in the fovea. The foveal fluorescein leakage observed in his eye resolved 10 days after sun gazing (Fig. 17).

Histopathologic study showed a spectrum of changes in these four patients. In the mildest case, the

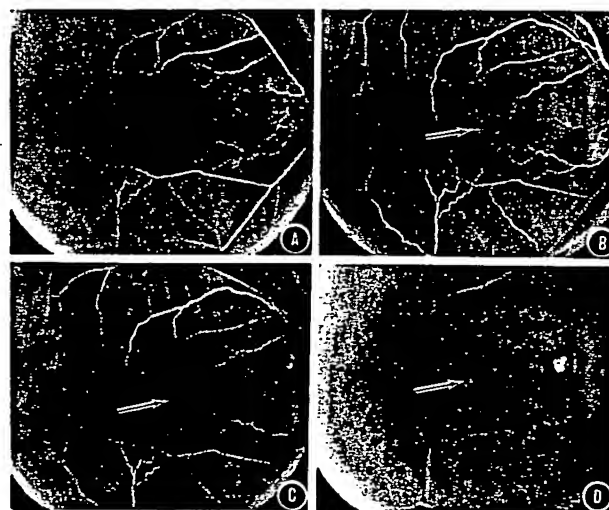


Fig. 16. Fluorescein angiogram of a patient who gazed at the sun. Note foveal leakage (arrows) 2 days after sun gazing in the late venous phase. From Tso MOM: Retinal Diseases. Philadelphia, JB Lippincott, 1988, pp. 187–214, by permission.

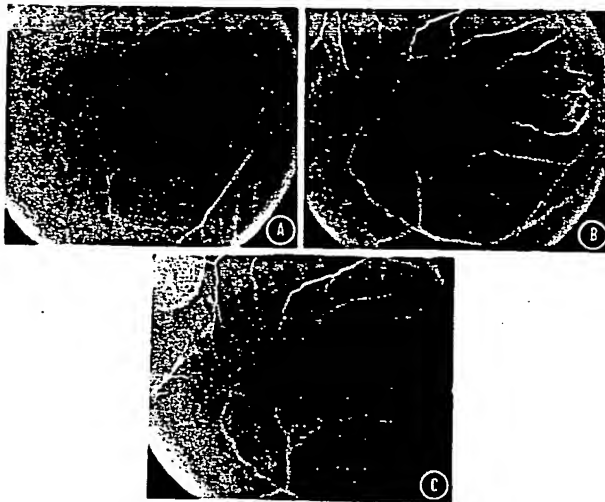


Fig. 17. Nine days after sun gazing, the foveal leakage shown in Figure 16 disappeared in all phases of the fluorescein angiogram. From Tso MOM: *Retinal Diseases*. Philadelphia, JB Lippincott, 1988, pp. 187-214, by permission.

retinal pigment epithelium demonstrated irregular depigmentation and margination of nuclear chromatin. Mild intracellular edema was observed in the retinal pigment epithelial cells without necrosis. Abundant phagosomes with engulfed photoreceptor outer segments and melanin granules were observed. In the more severe cases, the necrotic retinal pigment epithelium exhibited densified cytoplasm and disrupted plasma membrane and was sloughed into the subretinal space. At the edge of the foveal lesion, the retinal pigment epithelial cells were flattened and slid over bare Bruch's membrane. The pericytes of the choriocapillaris appeared to be activated and had migrated into Bruch's membrane. A shallow serous retinal detachment was observed, but the photoreceptor nuclei appeared unremarkable. The outer segments exhibited tubulovesicular changes, and densified aggregates of tubular structures were noted in the inner segments.

The patient whose eye was enucleated 12 days after sun-gazing showed a different histopathologic picture (Fig. 18). A thin layer of retinal pigment epithelial cells lined Bruch's membrane. Appearing in the subretinal space were pigment-laden macrophages clustered around damaged photoreceptor elements. A focal loss of photoreceptor nuclei was seen in the foveola.

These experiments suggested that sun-gazing could produce damage to photoreceptor cells and pigment epithelium. Since the patients experienced no pain on looking at the sun, they might have failed to detect any danger to their vision. The loss of visual acuity occurred 2 weeks after the exposure, and this delayed decrease in visual acuity might have prevented the patient under normal circumstances from linking the

light exposure to the loss of vision. While the earliest pathologic changes appeared in the retinal pigment epithelium, which was necrosed and sloughed off in the subretinal space, the photoreceptor showed only mild tubulovesicular changes of the outer segments—and microtubular aggregates in the inner segments, accounting for the initial good vision shortly after exposure. The retinal pigment epithelium regenerated rapidly, regaining the blood-retinal barrier. The photoreceptor cells, however, began to disappear and degenerate in the late phase; the mechanism of this delayed cell death is under active investigation in our laboratory.

The pathogenetic mechanism of solar retinopathy has been a subject of controversy. In 1916 Verhoeff and Bell<sup>65</sup> believed that solar retinopathy was photo-coagulative in nature and that the damage was caused by heat. The histopathologic features observed in our clinicopathologic study demonstrated no necrosis of

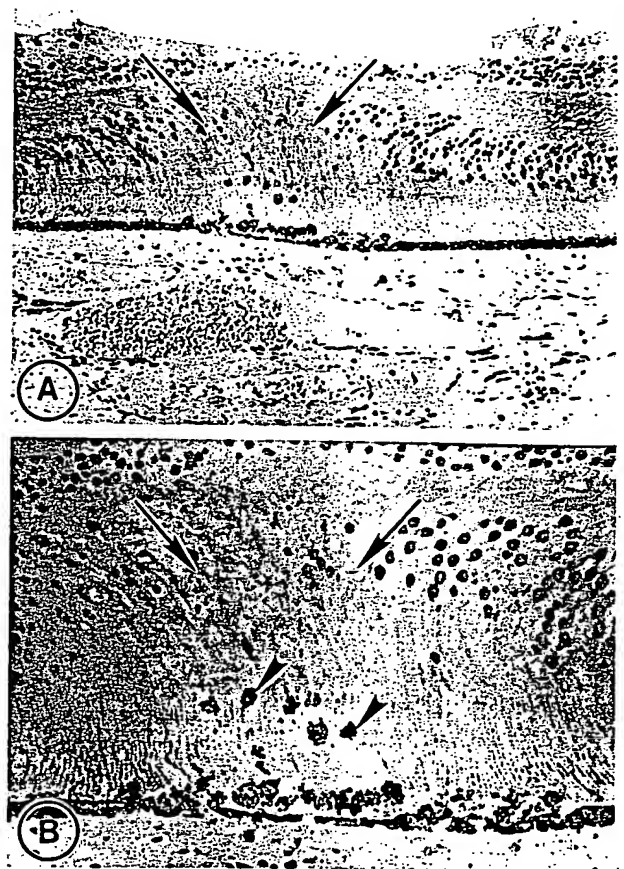


Fig. 18. Fovea of the patient shown in Figures 16 and 17. The eye was enucleated 12 days after sun gazing. (A) Fovea showed a distinct loss of photoreceptor cells (arrows) (toluidine blue,  $\times 100$ ). (B) Under high magnification, note the loss of photoreceptor cells (arrows), infiltration of subretinal space with pigment-laden macrophages (arrowheads), loss of photoreceptor elements and thin regenerated retinal pigment epithelium (toluidine blue,  $\times 225$ ). From Tso MOM: *Retinal Diseases*. Philadelphia, JB Lippincott, 1988, pp. 187-214, by permission.



photoreceptor cells, at least in the first 2 days after sun gazing. Vos studied solar retinopathy and showed that the retinal temperature rose only 2°C during sun-gazing.<sup>66</sup> Ham et al also believed that solar retinitis was primarily a photochemical event, because the temperature rise in the retinal lesion was insufficient to produce coagulative damage.<sup>67</sup> Most investigators believe that the pathogenetic mechanism of sun-gazing is photochemical in nature.

### Pathologic Process of Photic Retinopathy

The opportunities for human experimentation with photic retinopathy are limited. To determine the clinical course, the pathophysiologic parameters, the pathologic processes, and the physical characteristics of damaging light on the retina, photic retinopathy was studied in nonhuman primates.<sup>68-71</sup>

Photic retinopathy was induced in the macula of monkeys by exposure to the light of an indirect ophthalmoscope for 60-120 min with a +20-diopter condensing lens.<sup>68-71</sup> The clinical and pathologic changes were studied in three phases. No ophthalmoscopic changes were noted in the macula immediately after light exposure (phase 1). Mild edema and swelling of the retina developed 6-24 h later. Fluorescein angiography showed diffuse leakage of dye from the retinal pigment epithelium. Histopathologically, the retinal pigment epithelium appeared necrotic with disrupted plasma membrane, whereas the cell junctions between adjacent pigment epithelial cells frequently were intact. The disruption of the blood-retinal barrier was due mostly to the decompensation of the plasma membrane rather than to separation of the cell junctions. The photoreceptor outer segments showed marked tubulovesicular degeneration with moderate swelling of the Müller cell cytoplasm. The inner layers of the retina appeared unremarkable.

In the first month after exposure (phase 2) the retinal edema gradually subsided. Ophthalmoscopically, the macular area appeared spottily pigmented. An irregular staining of the retinal pigment epithelium was seen on fluorescein angiograms. The pigmented spots in the macula gradually diminished in size and disappeared after 3-5 months, leaving a yellowish white scar. Histologically, the retinal pigment epithelium was depigmented. Pigment-laden macrophages clustered around the damaged photoreceptor segments, accounting for the irregular pigmentation seen initially by ophthalmoscopy. In focal areas, the retinal pigment epithelium proliferated. The outer nuclear layer was thinned as a result of dropout of photoreceptor cells.

Two months to 5 years after light exposure (phase 3) the macula demonstrated a yellowish, raised, irregularly depigmented lesion. The arterial phase of fluo-

rescein angiography showed that the macular lesion had irregular hypofluorescence, but in the late venous phase, there was diffuse staining and leakage. Histologically, some of the proliferated retinal pigment epithelial cells appeared spindle-shaped, forming a placoid lesion. The spindle cells were surrounded entirely by basement membrane and had occasional junctional complexes between them. A layer of cuboidal retinal pigment epithelium overlay the plaque. In some animals, choroidal neovascularization extended into the retinal pigment epithelial plaque. The outer nuclear layer was reduced to one or two nuclei. The inner segments appeared plump, and the outer segments remained one third of the original length 5 years after exposure. The shortening of the outer segments seemed to be due to the impaired production of outer segment disks, since the number of phagosomes in the pigment epithelial cells did not appear to increase markedly. Horseradish peroxidase studies showed that tracer material leaked into the subretinal space and diffused forward to the external limiting membrane.

The severity of photic retinopathy in monkeys was studied further by varying the exposure time with an indirect ophthalmoscope from 30-120 min.<sup>71</sup> The photoreceptor cells appeared to undergo a morphologic staging of degeneration similar to that seen in age-related macular degeneration. In mild photic injury, the retinal pigment epithelium showed depigmentation with shrinkage of the nuclei, but the photoreceptor elements were aligned. In more severe injury with prolonged light exposure, the photoreceptors failed to produce outer segments 3-4 months after the injury. The outer nuclear layer may be reduced to a thickness of two to three nuclei. In even more advanced stages of degeneration, as seen in scorbutic cynomolgus monkeys after photic exposure, the inner segments were absent, and in focal areas, total loss of photoreceptor nuclei was seen. The exacerbated response in scorbutic animals to light damage will be discussed later. This pattern of photoreceptor degeneration is not unlike that seen in age-related macular degeneration (Fig. 15).

These experimental studies confirmed that there was a delayed degeneration of photoreceptor nuclei after photic injury. In mild injury, the rod and cone outer segments could regenerate. In more severe injury, the physiologic mechanism of outer segment production was impaired and the outer segments remained short. While the retinal pigment epithelium could quickly regenerate, disruption of the blood-retinal barrier might persist for years but did not appreciably interfere with visual function. Two monkeys with photic maculopathy were found by behavior study to have visual acuity of 20/30 to 20/40.<sup>70</sup> Subretinal neovascularization was produced in photic

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retinopathy, but did not show the aggressive growth as seen in human disciform macular degeneration. Pathologic features of photic retinopathy with graded severity did closely mimic those of age-related macular degeneration.

### The Role of Phototransduction Proteins in Photic Retinopathy

Since the phototransduction process is initiated at the photoreceptor cells, it is conceivable that in photic injury the proteins of the phototransduction process might be affected early in the degenerative process.

Edward et al examined the alteration of opsin, alpha- and beta-transducin, and arrestin (48K protein) in the rat retina after photic injury.<sup>72</sup> These antibodies were gifts from Paul Hargrave, Yee Kin Ho, and Nancy Mangini, respectively. Fifty-five-year-old male Lewis albino rats were exposed to 24 hr of green fluorescent light at 170–180 foot-candles. Immunolabeling of all four phototransduction proteins could be observed in the photoreceptor cells during the various stages of the ensuing degeneration. Six hours after photic exposure, apparent shifting of arrestin from inner segments to outer segments of photoreceptors during dark and light adaptation could still be demonstrated in the degenerating photoreceptor cells. In the visual cells with advanced degeneration in which the inner and outer segments had disappeared, opsin and transducins as well as 48K protein could still be immunolabeled in the perikaryon of the photoreceptor cells (Fig. 19). It appeared that even in severe photic injury the phototransduction proteins continued to be produced in the perikaryon of the photoreceptor cells.

### Physiologic Factors Affecting Photic Retinopathy

Our understanding of many anatomic and physiologic factors that influence photic injury has been derived from animal experiments in rats, rabbits, cats, dogs, pigeons, and nonhuman primates.<sup>64</sup> Some of these factors are species-specific. Others provide some generalized insight to the pathogenetic mechanisms of photic retinopathy.

Regional susceptibility of the retina to photic injury has been well documented.<sup>73–75</sup> The photoreceptors in the superior and temporal quadrants of the rat retina always sustained more severe injury than did the inferior and nasal quadrants. Rapp et al demonstrated that the difference in rhodopsin concentration between the superior and inferior quadrants of the rat retina is as great as 47%.<sup>76</sup> The retina, even that of a rat, is not a homogeneous organ. These regional differences in morphology, biochemistry, and physiolo-

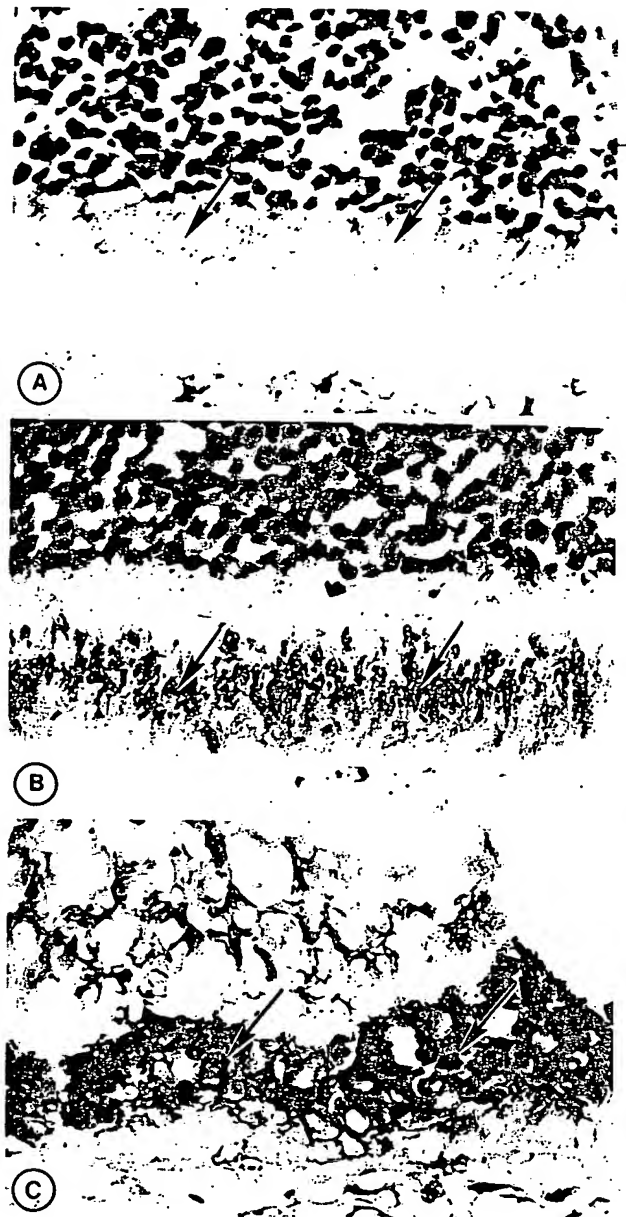


Fig. 19. In an albino rat exposed to green fluorescent light of 150 foot-candles for 24 hr, sections were incubated with 48K antibody. (A) Six hours after light exposure with the rat dark adapted, immunolabeling of 48K (arrows) was seen mostly in the inner segment and the outer nuclear layer. The rat was dark adapted. (B) Six hours after exposure with the rat light adapted, immunolabeling (arrows) was mostly seen in the outer segments. (C) Six days after exposure, loss of inner and outer segments was evident and the outer nuclear layer approached the retinal pigment epithelium. Immunolabeling (arrows) still present in the perikaryon of the photoreceptors showed continuous production of 48K protein despite severe photic injury.

ogy may provide clues as to why certain types of human photoreceptor degeneration occur preferentially in certain regions of the eye.

Light and dark adaptation affects the severity of retinal photic injury.<sup>77</sup> Rats maintained in cyclic

lighting are much more resistant to photic injury than are rats that are dark-adapted for 24 hr before light exposure. Increased body temperature also results in more severe retinal injury in rodents and monkeys after photic injury.<sup>78</sup> This observation may explain in part why highly febrile patients complain of photophobia. Pigmentation of the iris and retina affects the severity of retinal photic injury.<sup>79</sup> It has long been suspected that albino patients, who frequently are photosensitive and have central scotoma, may be more susceptible to photic injury than are normally pigmented persons. However, when Rapp and Williams<sup>79</sup> exposed albino and pigmented rats with dilated pupils to light, which was controlled to produce equal steady-state bleaching in both strains of rats, they observed comparable retinal degeneration in both. These investigators believed that the pigmented rats appeared less susceptible to light damage because iris pigmentation lowered the retinal irradiation.

Rats, rabbits, frogs, pigeons, dogs, and humans exhibit marked differences in susceptibility to photic injury. Rabbits, monkeys, and humans have a much higher threshold to photic injury than rats. Hamsters suffered more damage when exposed to light than did cats and rabbits.<sup>80</sup> Light damage appeared to increase with advancing age in rats.<sup>81</sup> Susceptibility to light damage also varied among different strains of the same species. La Vail and colleagues demonstrated variation in photic susceptibility among albino mice of different strains.<sup>82</sup> We studied the light susceptibility of four strains of albino rats, including Fisher, Buffalo, Lewis, and Wister rats (unpublished data, 1989). Lewis rats were more susceptible to photic injury than were the other strains. In another study, rats deficient in vitamin A also showed more resistance to light damage than did rats fed a normal diet.<sup>83</sup> This observation supports the hypothesis that photic injury in rats is rhodopsin-dependent. Short-wavelength light appeared to produce more severe retinal injury than did long-wavelength light.<sup>84-88</sup> In general, the action spectrum of the damaging light is a function of the decreasing wavelength.

Different schedules of light exposure result in different degrees of photic injury. Lee et al compared the retinal photic injury in 3 groups of rats<sup>89</sup>: (1) rats exposed to continuous green-filtered fluorescent light at 150-170 foot-candles; (2) rats exposed to repeated light/dark cycles of 10 sec; and (3) rats exposed to repeated light/dark cycles of 90 min. Histologically, the 90 min on-and-off schedule inflicted the maximum photic injury, while the 10 sec on-and-off schedule resulted in mild degeneration of pigment epithelium and photoreceptor elements (Fig. 20). Furthermore, the 10 sec on-and-off exposure group preserved the highest level of rhodopsin in the retina;

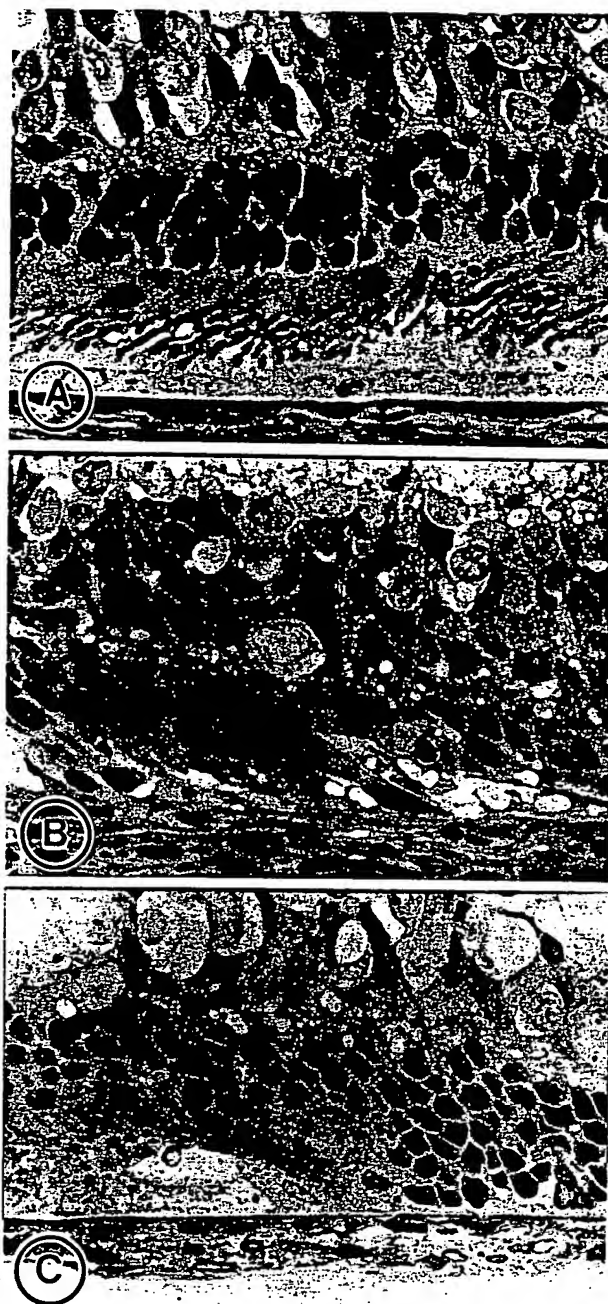


Fig. 20. Rats exposed to green fluorescent light at 170 foot-candles for a total light period of 24 hr on different exposure schedules. (A) The retina exposed to a 10-sec-on, 10-sec-off schedule showed thinning of the photoreceptor layer but preservation of the retinal pigment epithelium. (B) Retina exposed to a 90 min on-and-off schedule showing extensive loss of photoreceptor elements and pigment epithelium. (C) Retina exposed to a continuous schedule, showing loss of photoreceptor cells and elements and focal loss of retinal pigment epithelium.

the continuous exposure group had an intermediate level; and the 90 min on-and-off exposure group had the least. These observations suggested that a comparable total-energy dose inflicted a varying severity of retinal photic damage with different exposure sched-

ules. These findings are also in line with those of Sperling and co-workers, who observed that intermittent blue and green light produced damage to blue and green cone cells in monkeys, while continuous exposure created more severe damage to the retinal pigment epithelium.<sup>84</sup>

### Proposed Mechanisms of Retinal Photic Injury

The pathogenic mechanisms of photic retinopathy probably consist of a cascade of reactions. Noell<sup>74</sup> suggested that there were two kinds of photic injury. The first was characterized by loss of pigment epithelium and photoreceptor nuclei. A second kind of damage was described in rats younger than 24 days old that were exposed to continuous light for 8–50 days. The retinal damage produced was characterized by widespread loss of photoreceptor cells, but the retinal pigment epithelium was preserved. Noell also suggested that photooxidation may be an important mechanism of photic injury. Feeney et al<sup>90</sup> proposed that photic exposure might produce a series of free radicals that react with unsaturated fatty acids in the photoreceptor–retinal pigment epithelium complex and then result in lipid peroxidation. Lawwill<sup>91</sup> summarized three pathophysiologic mechanisms of retinal photic injury. The first was rhodopsin-specific; the second was cone-pigment-specific; and the third mechanism involved direct action of blue light on the retinal tissue, resulting in damage to all layers of the retina.

In our study of photic injury in monkeys, three distinct phases of tissue response were delineated,<sup>71</sup> and each phase involved different pathologic mechanisms. In the initial 48 hr of light exposure, the pathologic changes were confined to disruption and vesiculation of the photoreceptor outer segments, vacuolation of the mitochondria in the inner segments, and swelling of the retinal pigment epithelium. Melanin granules in the retinal pigment epithelium absorb some of the excess energy and serve as a scavenger of light-induced free radicals. In severe photic injury, melanin granules themselves become cytotoxic, are surrounded by necrotic cytoplasm, and are engulfed within the phagosomes of retinal pigment epithelium.

In the highly oxygenated environment of the outer layers of the retina, where excessive light energy is adsorbed by a number of retinal photosensitizers, such as riboflavin, retinal, and cytochrome *c*, a photodynamic reaction takes place. As the photosensitizers absorb the excessive energy, they are elevated to the singlet and later to the triplet state. The singlet state is short-lived and dissipates energy into a long-lived triplet state in high quantity. Photosensitizers in the triplet state may undergo types I or II reactions. In

the type I reaction, the activated photosensitizers react with substrate in the tissue, producing free radicals. In the type II reaction, the photosensitizers in the triplet state react with oxygen, forming singlet oxygen. This reaction accounts for most of the quenching of the photosensitizers in the triplet state. A small amount of superoxide radicals may also be generated. In these reactions, the free radicals and singlet oxygen may react with polyunsaturated fatty acids in the photoreceptor cells and retinal pigment epithelium, resulting in lipid peroxidation and tissue damage.

In the second phase of retinal photic injury, macrophages from systemic circulation invade the subretinal space to clean up the cellular debris. In the early phase of photic injury, especially in rats, there could be a loss of 25% of photoreceptor nuclei without the appearance of macrophages. Some cellular debris was observed within Müller cells. The exact mode of the dissolution of photoreceptor nuclei needs further investigation. The chemotactic agent for macrophagic infiltration is still a subject of speculation. A possible candidate may be leukotriene, a breakdown product of arachidonic acid, which is present only in small quantities in the photoreceptor elements.

The macrophages initiate a different set of destructive mechanisms. These activated phagocytes start a respiratory burst in which a 10-fold–20-fold increase of oxygen is consumed, producing a series of oxygen-free radicals, including superoxide radicals and hydrogen peroxide. In the presence of iron, the superoxide radicals and hydrogen peroxide generate hydroxyl groups through the well-known reactions of Haber-Weiss and Fenton. With these free radicals, the macrophages digest cellular debris and even some of the normal photoreceptors. This hypothesis appears to be consistent with the observations that after sun-gazing our patients had relatively good visual acuity and that the photoreceptor cells started to degenerate as macrophages started to appear.

A third phase of retinal photic injury consists of chronic degeneration. Macrophages remain in the subretinal space as long as 5 years after an initial insult, and the photooxidative reaction lingers on. The nuclei of the photoreceptor cells gradually drop out, and the outer segments become short and stubby and, in severe injury, eventually fail to form.<sup>49,71</sup> The cellular mechanisms of physiologic renewal of outer segments is likely to have been damaged. The retinal pigment epithelium appears thin and flat. Disruption of the blood–retinal barrier persists as long as 5 years after the initial photic damage. The retina slips into the chronic degenerative phase, which is characterized by pathologic features not unlike those seen in age-related macular degeneration.

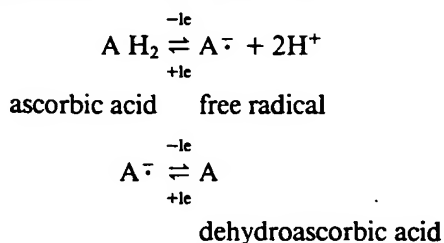


The mechanism of the delayed photoreceptor-cell death after light exposure, so well demonstrated in the human sun-gazing experiments, is not known. L-glutamate has been proposed to be a neurotransmitter for photoreceptor cells. Recent evidence suggests that the release of glutamate-related excitatory amino acids may cause an influx of calcium into cellular elements.<sup>92</sup> Calcium has been suggested to be related to delayed neuronal death by activating protease and lipase. We have observed calcium deposition within the mitochondria and cytoplasm of photoreceptor cells during photic injury in rats, particularly in the retina exposed to a schedule of on-and-off light. This hypothesis may link the phototransduction process to photoreceptor cell necrosis.

### A Therapeutic Approach to Photoreceptor Degeneration in Photic Retinopathy

#### Ascorbate in Photic Retinopathy

In search of therapeutic agents that may ameliorate photic injury, we examined the effects of ascorbate on retinal photic injury. Ascorbate is a reducing agent which is present in an abundant amount in the retina. It has a redox potential of +0.08 V for reducing dehydroascorbic acid to ascorbic acid.



One of the unique features of the ascorbate system is that its free radical is stable and nonreactive and may decay disproportionately to ascorbic and dehydroascorbic acid. As the ascorbate-free radicals react preferentially with themselves, the propagation of free radical reaction is terminated.<sup>71,93</sup>

With high-pressure liquid chromatography we observed that 97% of the total ascorbate in the neuroretina of guinea pig was in reduced form.<sup>94</sup> After mild photic insult, the reduced ascorbate decreased in the neuroretina, and the oxidized ascorbate increased. Further investigation of photic injury in baboons demonstrated that 93% of the total ascorbate in the normal neuroretina was in reduced form.<sup>95</sup> After exposure of the baboon retina to the light of a direct ophthalmoscope for 0.5 h, the reduced ascorbate of the neuroretina decreased. In another experiment, Organisciak and co-workers exposed normal rats of different age groups to fluorescent light for 24 hr and demonstrated that the retinal ascorbate significantly decreased in most of the age groups.<sup>96</sup> These results suggested that the abundant reduced ascorbate in the

neuroretina of guinea pigs, baboons, and rats may act as an antioxidant and is oxidized by free radicals generated during excessive light exposure.

Woodford and Tso<sup>97</sup> compared the photic injury between normal and scorbutic guinea pigs and observed that the scorbutic animals suffered more severe retinal injury. Organisciak and associates, in collaboration with our laboratory, exposed rats that were given a normal diet or a diet supplemented by vitamin C, to green fluorescent light.<sup>48,98</sup> Ascorbic administration reduced the loss of rhodopsin after photic exposure and strongly suggested that ascorbate offered protection against retinal photic injury. L-ascorbic acid, sodium ascorbate, and hydroascorbate were equally effective in preventing rhodopsin loss after photic injury, while D-ascorbate was not. The protective effect of rhodopsin loss by ascorbate was also dose-dependent. However, relatively large doses (0.2–0.25 mg/kg of body weight) were required for optimal protection. Ascorbate was effective only when administered before light exposure and not afterward. Morphometric studies of the remaining photoreceptor nuclei in the rat retina after light exposure showed that rats given ascorbate supplements had substantially less retinal damage. Morphologically, rats with vitamin C supplements showed better preservation of retinal pigment epithelium<sup>48</sup> (Fig. 21). Noell et al interpreted these changes as a shifting from type 1 retinal damage to type 2 retinal damage by ascorbate administration,<sup>99</sup> and further proposed that ascorbate may specifically protect retinal pigment epithelium against a toxic product or that a pigment-epithelium-produced toxin in light damage is reduced after the administration of ascorbate.

Comparable study on photic retinopathy was conducted in scorbutic monkeys by Tso<sup>71</sup> by inflicting mild or severe photic injury (30 min or 120 min of light exposure, respectively) on seven monkeys fed on a vitamin-C-deficient diet and four monkeys given a vitamin-C-enriched diet. Clinically, the fundus of the normal monkey eyes after exposure to the light of an indirect ophthalmoscope showed mild edema with moderate leakage of fluorescein, but the retinas of scorbutic monkeys showed considerably exaggerated retinal edema and fluorescein leakage. Although the general cellular response to the photic injury in the retina of scorbutic animals was not different qualitatively from that of the normal animals, scurvy appeared to cause additional tissue damage, an exaggerated repair response, and more severe retinal degeneration. By electron microscopy of the four groups of eyes representing mild or severe photic injury in normal or scorbutic animals, a continuous spectrum of pathologic changes was observed. The least damage occurred with mild photic injury in the normal ani-

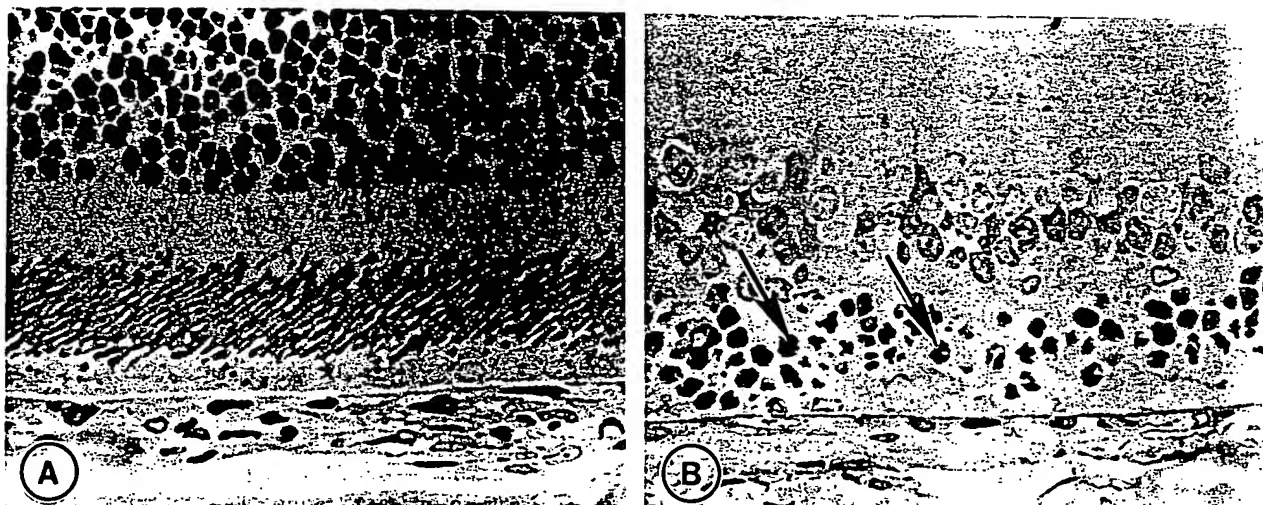


Fig. 21. (A) Retina of a rat given large supplements of vitamin C and then exposed to fluorescent light for 24 hr. Note the preservation of photoreceptor elements and pigment epithelium (toluidine blue,  $\times 370$ ). (B) Retina of a rat fed with a normal diet but no vitamin C supplement was exposed to the same light. The retina showed loss of photoreceptor elements and nuclei (arrows) and focal loss of pigment epithelium (toluidine blue,  $\times 320$ ). From Tso MOM: *Ophthalmology* 92:628, 1985, by permission.

mals, and the most detrimental insult resulted from severe photic injury in the scorbutic animals.

We propose that the basic mechanism by which ascorbate mitigates retinal photic injury depends on its redox properties. As a scavenger of superoxide radicals and hydroxy radicals, it also quenches singlet oxygen and reduces hydrogen peroxide, all of which probably are formed in retinal photic injury. This hypothesis provides an explanation of a high level of naturally occurring ascorbate in the normal retina.

It has been observed that aging has been associated with subclinical scurvy.<sup>100</sup> This subclinical scurvy may lead to greater susceptibility of the retina to photic injury. In some forms of age-related macular degeneration, the patients may suffer from exaggerated photic insult. Similarly, smokers<sup>101</sup> have exhibited subclinical scurvy and may show an earlier development of age-related macular degeneration than do nonsmokers with the disease. Although ascorbate moderates many biochemical functions of the body and helps the retina ameliorate photooxidative injury, it should be regarded as a nutritional supplement to maintain health when consumed in appropriate amounts. Vitamin C is quickly excreted through the kidneys, and it is difficult to maintain high levels in the retina.

#### Beta-Carotene in Photic Retinopathy

In our search for other therapeutic agents that may ameliorate retinal photic injury, we examined the protectors of photodynamic reaction in the plant and animal kingdoms. The carotenoid pigments are present widely among photosynthetic organisms in na-

ture.<sup>102-106</sup> Foote<sup>107</sup> demonstrated that carotenoid pigments has the capability of physically quenching singlet oxygen generated during photosynthesizing reactions. Beta-carotene and other carotenoids with nine conjugated double bonds have been shown to be the most efficient agents to quench the excitation of triplet photosensitizers and singlet oxygen. Krinsky and Deneke<sup>105</sup> proposed that carotenoids can, in addition, chemically react with singlet oxygen and react with radical intermediates. They also demonstrated that the carotenoid pigments are capable of inhibiting lipid peroxidations. Carotenoids such as leutin and zeaxanthin are constituents of the yellow pigment of the normal macula lutea of human.<sup>108,109</sup> Ham and co-workers fed one monkey with beta-carotene for 64 days and noted that the threshold of minimal retinal damage from blue light was elevated by approximately 44%.<sup>110</sup>

To investigate the possible use of beta-carotene in ameliorating photic injury, we established that a single intraperitoneal injection of beta-carotene at a dose of 35 mg/kg of body weight in rats could bring the average concentration of beta-carotene in the retina to about 0.4  $\mu\text{g}/\text{ml}$  wet tissue at 24 hr after injection.<sup>111</sup> The elimination process of beta-carotene from the retina was biphasic. On the first 4 days, the half-life was 2 days, and was followed by a 7 day half-life.

To study the ameliorative effect of beta-carotene on retinal photic injury, we gave each of 13 rats four intraperitoneal injections of beta-carotene at a dose of 35 mg/kg. Another 13 rats formed the control group. The rats were exposed to continuous green-filtered fluorescent light with an intensity of 220-250

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foot-candles for 24 hr. The photic injury was studied by morphology, morphometry, and rhodopsin measurements of the retina as indices of photic injury. Six hours after light injury there was considerable loss of photoreceptor nuclei in both the control and treated rats. Six to 14 days after light exposure there was a dramatic difference between the controls and the treated animals, with the treated animals being less affected. The treated rats demonstrated better preservation of the photoreceptor nuclei and retinal pigment epithelium in all quadrants (Fig. 22). The rhodopsin measurements of the treated and control retinas supported the morphologic and morphometric results. Beta-carotene indeed ameliorated photic injury in the rat retina. This observation further confirmed our hypothesis that the photodynamic reaction is part of the mechanism of retinal photic injury and that singlet oxygen and oxygen-free radicals play an important part in retinal damage. However, the initial loss of photoreceptor nuclei 6 hr after light exposure, when the treated and control rats showed comparable morphologic changes, suggested that other mechanisms also were involved and need to be explored further.

To seek clues about the beneficial effects of beta-carotene on age-related macular degeneration, our group, headed by Dr. Jack Goldberg,<sup>112</sup> reanalyzed the 1971-1972 data from the first National Health and Nutritional Examination Survey. About 10,000 subjects from the nutritional survey had eye examinations. We noted that the frequency of consumption of fruits and vegetables rich in carotenoid and vitamin A is negatively associated with prevalence of age-related macular degeneration, even after adjustment for demographic and medical factors.

### Summary

Until recent years we have had limited options in our treatment for photoreceptor degeneration. The therapeutic approach has been simply mechanical in nature, having consisted of surgical apposition of detached photoreceptors to pigment epithelium, and laser ablation of leakage from retinal pigment epithelium and choroid. No medication is available. Our experiments with vitamin C and beta-carotene in photic retinopathy have initiated a new therapeutic approach to the treatment of photoreceptor degeneration. My analysis of the morphologic, pathophysiologic, biochemical, and pharmacologic response of the retina to photic retinopathy leads me to believe that antioxidants play an important part in the pathologic process of photic retinopathy, and will ameliorate the degenerative process. Different pathogenetic mechanisms probably affect different phases of the

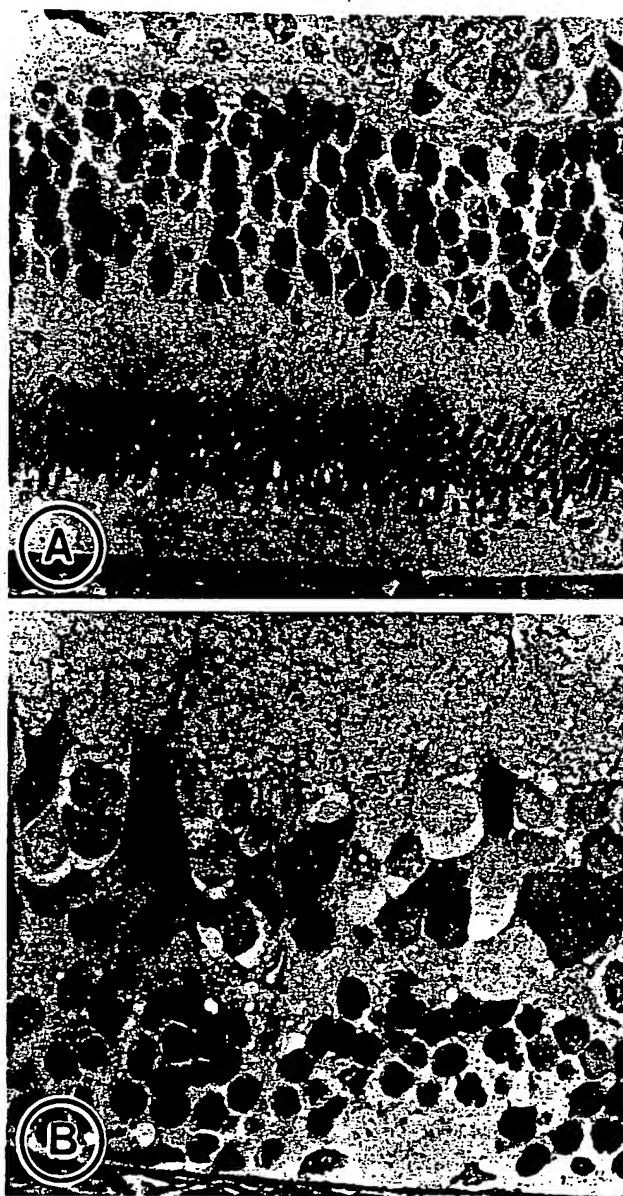


Fig. 22. (A) Retina of a rat given beta-carotene and then exposed to green fluorescent light for 24 hr. Note the preservation of photoreceptor elements and pigment epithelium. There was thinning of the outer nuclear layer. (B) Retina of a rat fed with normal diet but no beta-carotene was exposed to the same light. The retina showed marked loss of photoreceptor cells and elements and focal loss of pigment epithelium.

degenerative processes. Antioxidants are effective in the early phase. Other therapeutic agents are being tested to influence different phases of the degenerative process. New experimental models of photoreceptor degeneration with viral, immunological, ischemic, aging, and hereditary pathogenetic factors are being developed so that a more comprehensive understanding of photoreceptor degeneration may be achieved. With this approach, the cloud of mystery surrounding photoreceptor degeneration will soon be

dispersed, and new medical treatment of photoreceptor degeneration will be on the horizon.

### Acknowledgements

The author thanks Drs. Deepak Edward and Tim Tak Lam for comments; Ms. Kathleen Loudon for editing; and Ms. Betty Gordon, Mary Imhoff, and Bettie Ann Cleveland for preparation of the manuscript.

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